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# Comprehensive Contribution of Filament Thickness and Crosslinker Failure to the Rheological

## Property of F-actin Cytoskeleton

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**Abstract:** Rheological property of F-actin cytoskeleton is significant to the restructuring of cytoskeleton under a variety of cell activities. This study numerically validates the rheological property of F-actin cytoskeleton is not only a result of kinetic energy dissipation of F-actin, but also greatly depends on the configuration remodeling of networks structure. Both filament geometry and crosslinker properties can affect the remodeling of F-actin cytoskeleton. The crosslinker unbinding is found to dissipate energy and induce prominent stress relaxation in the F-actin adjacent to cross-linkages. Coupled with F-actin elasticity, the energy dissipation and stress relaxation are more significant in bundled F-actin networks than in single F-actin networks.

**Keywords:** F-actin networks, Crosslinker, Energy dissipation, Mechanics

## 1. Introduction

One of the most important characteristics of living eukaryotic cells is that they can adapt their mechanical properties at multiple timescales to best respond to external stimuli <sup>1,2</sup>. Such changes of cellular mechanical properties are underpinned by the mechanobiological response of the cell cytoskeleton, a complex network consisting of thick (microtubule), intermediate (intermediate filament) and thin (F-actin) biological filaments. Among these cytoskeleton components, actin has been identified to serve a critical role in the mechanical responses of living cells <sup>3,4</sup>. The mechanical toughness of living cells is collectively fulfilled by the high tensile stiffness of single F-actin which is typically at the GPa scale <sup>5</sup>, and the rheological properties of actin gels which maintain the flexibility of living cells at long timescales <sup>6</sup>. This adaptability of cells with respect to timescale is achieved by complex mechanosensing rearrangements of F-actin cytoskeleton, which is a composite network with constantly changing chemical components. Various functional and structural proteins work in conjunction to archive the phase change of cells under physical forces, which is significant to their self-protective properties in chemical environments.

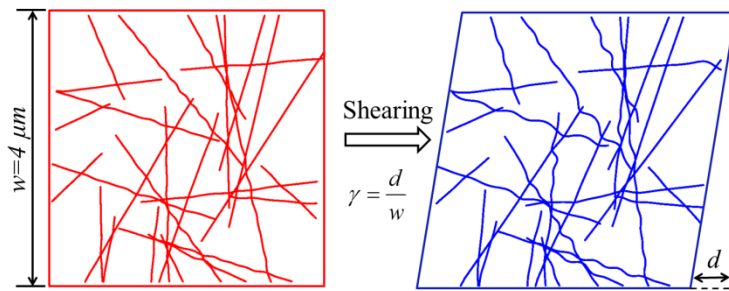
The underlying structural rearrangements in living cells can be promptly accelerated once the cells are exposed to physical forces. Subsequent cell relaxation would result in a significant phase change <sup>7</sup> in which the local stress relaxation of cellular structure plays critical roles. This cryptic physiological phenomenon is known to depend on the remodeling properties of F-actin cytoskeleton. Dynamically synthesized by cytoplasm <sup>8</sup>, the F-actin in living cells has multiple states varying from single filaments to filament bundles <sup>6</sup>. It has been demonstrated that, in bundled F-actin networks, the potential energy slowly dissipates with the structural remodeling of the networks <sup>9</sup>. Compared with single F-actin networks, the F-actin bundle networks feature enhanced stiffness and strength, both favored for living cells to resist transient mechanical deformation.

As well known, one of the most typical characteristics of tumor cells is their different mechanical stiffness compared with the normal ones <sup>10,11</sup>. These mechanical properties change can reflect the health of living cells. It is crucial for a living cell to mediate its stiffness and releasing the deformation energy induced by mechanical inputs. A previous experiment treated isotropically crosslinked actin networks with glutaraldehyde to fix the binding interaction between actin and heavy meromyosin, and discovered that the critical ability of energy dissipation was significantly diminished <sup>12</sup>. Crosslinker unbinding events were therefore proposed to be responsible for the temporal, spontaneous stress relaxation in bundled F-actin networks. Despite the progress in the observation of actin networks remodeling at microscale <sup>9</sup>, the resolution of *in-situ* experiments is still limited to directly characterize the molecular events happening during the structural remodeling of F-actin networks. To improve the understandings of the important dissipative properties of F-actin networks, a granular modelling strategy is developed to explicitly track the dynamical behaviors of F-actin networks in association with crosslinkers. This granular modelling strategy has been adopted to study the compression of F-actin CSK and explained the significance of transient crosslinker failure to the mechanical deformation properties of CSK <sup>13</sup>. However, the temporal scale in this granular model is limited by today's computer power and it is impossible to fully track the physiological process of CSK remodeling,

which happens at the time scale of hours. In this paper, we putatively fixed the crosslinkers in CSK to achieve relatively stable configuration and then suddenly allow the crosslinkers to fail. The transient remodeling of networks is caused by the sudden crosslinker failure. The potential energy evaluations are quantified with respects to filament thickness to understand the contribution of fiber properties in the networks.

## 2. Model

In the present investigation, we have developed an actin networks with isotropic configuration of F-actin network. This is similar to the experimental characterization by Lieleg, et al <sup>9</sup>, in which F-actin bundles are isotropically oriented (Fig. 1). A prescribed shear strain  $\gamma$ , ranging from 0.05 to 0.2, is applied to the  $4\mu\text{m} \times 4\mu\text{m}$  network to mimic the boundary constraints in rheology measurements. Assuming the line elements in Fig. 1 to be single F-actin filaments or F-actin bundles generates two models, i.e. a single F-actin network model and a bundled F-actin network model. The F-actin bundle in this study is considered to have 10 parallel single filaments, which is a fair assumption based on both experimental and theoretical studies <sup>14,15</sup>. It needs to be noted that, the filament length in this network model is not strictly controlled and the length ranges from a few hundreds nanometer to 4 micrometer. In the granular modeling, every two adjacent G-actin monomers in a single filament are merged and simplified as one bead. The same model is also employed for modelling the bundled F-actin, where the harmonic elasticity is increased by 10 times to reflect the enlarged cross-section. It should be noted that, there are also crosslinker inside each F-actin bundle. However, due to the computational cost, the effects of bundle state are implicitly presented by the mechanical parameters changes. From the viewpoint of mechanics, the internal crosslinkers are of less chance to fail comparing to those crosslinkers connecting different F-actin bundles, as stress intensity usually happens near the connection. As a result, these crosslinkers are assumed to be fixed in the process of deformation to simplify the calculation. The timescale resolution is controlled as 0.1ps to fully extract the trajectory of macromolecules. More details about the model and simulation parameters can be found in supplementary material.



**Fig. 1 Shear deformation of the F-actin network in modelling**

During the simulations, crosslinkers are allowed to form when any two G-actin clusters (from different filaments) are separated less than 20nm. The transient crosslinker unbinding takes place when the force on the cross-linkage exceeds a threshold of  $\sigma_{ult}$ . We have designed different modellings considering this ultimate force as constant, Gaussian distribution and uniform distribution. It has been found that, the stochasticity has effects on the mechanical performance of cytoskeleton (CSK).

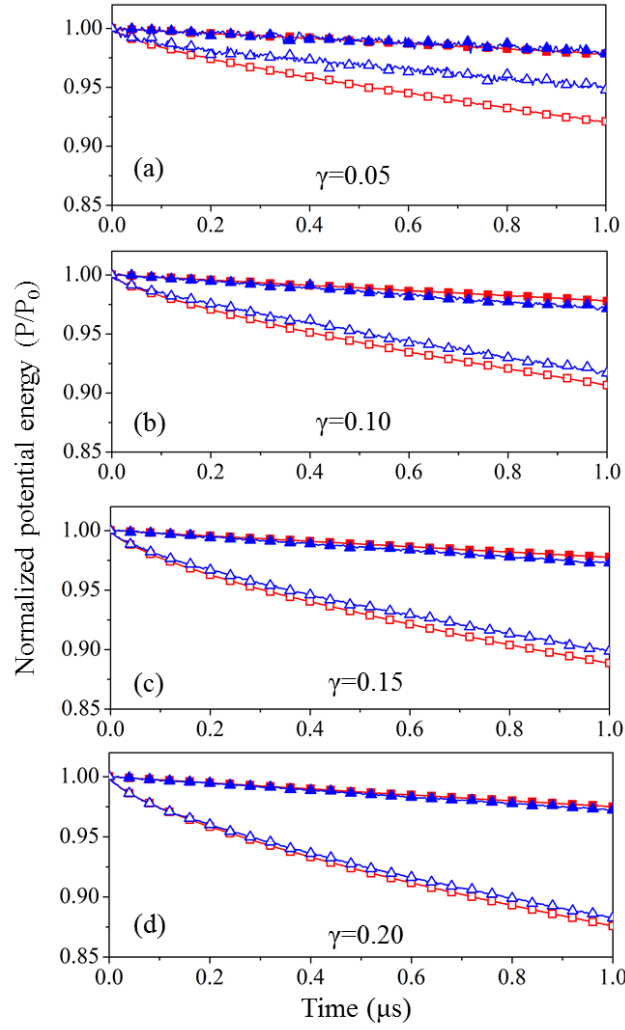
However, the difference between uniform distribution and Gauss distribution is not significant. Hence, this ultimate strength is assumed to follow Gauss distribution of  $\sigma_{ult} \sim N(180,10)$  in this paper, which is at the level of many experimental findings such as  $\alpha$ -actinin unfolding<sup>16</sup> and filamin unbinding<sup>17,18</sup>. The comparison between different strength distributions can be found in the supplementary material (Figs. S3 and S4).

The sheared F-actin networks are first relaxed by geometric optimization and 20 $\mu$ s granular dynamics simulations (temperature of 300K) in which the crosslinkers are not allowed to fail. This 20 $\mu$ s system relaxation is just numerical relaxation for us to obtain a relatively stable molecular configuration of actin networks that can be used for later dynamics simulation. After the system relaxation, network models are differently treated regarding crosslinker conditions and these dynamics simulations last for another one micro-seconds to study the evolution of stress and energy with respects to different cross-linkage conditions. For the purpose of comparison, two cases are considered for each of the modelled F-actin networks. In the first case, the crosslinkers are allowed to form and break when the corresponding criteria be satisfied, which mimics the real physical processes. In the second case, crosslinkers are not allowed to fail from the network, which simulates F-actin networks treated with glutaraldehyd<sup>9</sup>. The impact of crosslinker failure can be then reflected by the difference of those dissipation curves due to different crosslinker treatments. It should be noted that, even 20 $\mu$ s takes long computation time to accomplish, it is still not long enough for a fully system relaxation of this nature event. Slight energy dissipation still happens after the 20 $\mu$ s relaxation, as is provided in supplementary material (Figs. S2). However, we only focus on the difference between these curves to understand the significance of crosslinker dynamics in the stress relaxation of F-actin CSK.

The micro-second level simulations performed in this study are enabled by the highly coarse-grained granular modeling of the F-actin networks. The large timescale is very important for capturing time-dependent material properties such as stress relaxation. Moreover, by not allowing the crosslinkers to fail during the relaxation, we exclude the loading-rate effect so the present computational study can mimic the quasi-static loading in experiments.

### 3. Results and discussion

With the aforementioned explicit modelling strategy, we are able to capture the potential energy carrying ability of F-actin networks with both detachable and non-detachable crosslinkers. According to conventional thermostat theory, the kinetic energy only have dependency on the temperature, therefore, only potential energy of the network is tracked in this study to understand the energy evolution in time domain. Bundled F-actin networks and single F-actin networks have been independently studied to investigate the effects of filament thickness on the dissipative properties of F-actin networks. The profiles of potential energy in different modeling scenarios are provided in Fig. 2. All values are normalized by the initial potential energy before stress relaxation. The normalized potential energy serves as a quantitative characterization of the energy dissipation in the network.

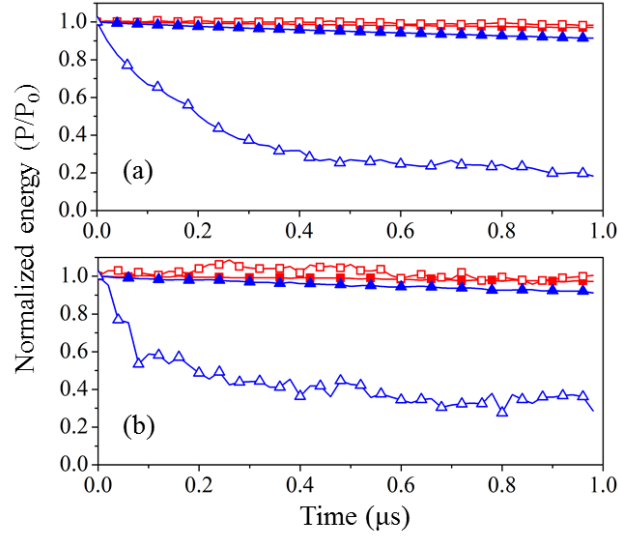


**Fig. 2** The energy dissipation in F-actin networks under different deformation conditions. (a) to (d) relatively represent the situation of shear strain ( $\gamma$ ) from 0.05 to 0.2. In all these sub-figures: red solid square denotes bundled F-actin networks with non-detachable crosslinkers; red open square is the bundled F-actin networks with detachable crosslinkers; blue solid triangle is single F-actin networks with non-detachable crosslinkers; blue open triangle is single F-actin networks with detachable crosslinkers.

From the modelling results, neither bundled nor single F-actin networks show significant energy dissipation when crosslinker unbinding is putatively prohibited. The dissipation of deformation energy in F-actin networks is the reflection of cellular remodeling. This inconspicuous change of energy indicates that networks with rigid crosslinkers are insufficient to release the stress caused by mechanical inputs. However, when crosslinkers are allowed to unbind, the energy dissipation rate can be 3-8 times higher, leading to about 10% energy dissipation in  $1\mu s$ . We note that, the energy dissipation mainly happens on F-actin filaments or bundles, not on the crosslinkers. For example in the simulation of single filament networks under shear strain of 0.1, the energy dissipation in the  $1\mu s$  simulation is 5601.88 kcal/mol. However, the change of potential energy stored in crosslinkers is only 94.49 kcal/mol. Therefore, the energy dissipation is more related to the structural rearrangement of CSK networks, instead of the direct loss of potential energy in the crosslinkers.

In the whole shear strain range ( $\gamma=0.05\sim 0.2$ ), bundled F-actin networks exhibit higher energy dissipation compared to the networks of single filaments. The rate of energy dissipation has proportional dependency on the shear deformation. The more F-actin network deforms, the more deformation energy will be released by the material in unit duration. However, the rate of

energy dissipation in single F-actin networks would approach bundled F-actin networks with the increase of shear deformation. We have independently extracted the potential energy carried by F-actin and crosslinkers to further explain these specific characteristics of F-actin cytoskeleton. The separated potential energy is also normalized with respects to the initial energy state for the purpose of comparison, as shown in Fig. 3. Without loss of generality, we only tracked the energy profiles for the case with a shear strain of 0.05.



**Fig. 3** The energy separation for F-actin and crosslinkers when  $\gamma=0.05$ . (a) represents a network with bundled F-actin; (b) represent a network of single filaments. In all sub-figures: red, solid square and red open square respectively denotes the energy carried by F-actin and crosslinkers on a network with non-detachable crosslinkers; blue, solid and open triangle respectively denotes the energy carried by F-actin and crosslinker on a network with detachable crosslinkers.

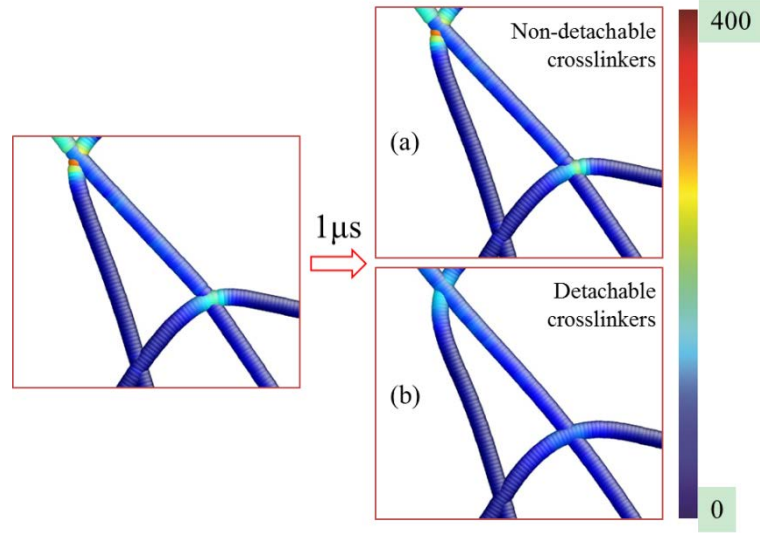
Neither of the deformation energy carried by F-actin or crosslinker shows significant energy dissipation phenomenon when the crosslinker unbinding events are prohibited, which is consistent with conclusions from the energy profiles in Fig. 2. For bundled F-actin network, the potential energy carried by crosslinker reduces promptly after crosslinkers are allowed to fail in the network. This energy deduction physically illustrates that the crosslinkers are unbinding from F-actin. As the mechanical constrains (cross-linkage) in the network structure are removed due to the unbinding events, the network is more flexible to self-remodel and release the concentrated deformation energy stored near F-actin cross-linkage. Compared to networks with detachable crosslinker, the network with non-detachable crosslinkers lacks this flexibility to remodel its structure in response to external mechanical signals.

When the network is structured by single filaments, the potential energy carried by crosslinkers also has a chance to be released. However, due to the low stiffness of the element in single F-actin networks, the stress-level at cross-linkage would not be as significant as that in bundled F-actin networks. The lower stress-level promises that the deflection of filaments on the network would be flexible and not as sensitive to limited deformation as bundled F-actin networks. Correspondingly, the crosslinkers still reserve the possibility to rebind on actin after temporary unbinding, which is the reason of the increasing zigzag energy profile of crosslinkers. However, with the increase of deformation, the stress-level would be amplified at the cross-linkage, making the unbinding events of crosslinkers more irreversible and the energy releasing behaviors of single F-

actin networks would approach that of bundled F-actin networks. When the strain is large ( $\gamma=0.2$ ), the deformation energy carried by crosslinkers in single F-actin networks also present monotonous downtrend, instead of the zigzag profile when the strain is small ( $\gamma=0.05$  or  $\gamma=0.1$ ). This also explains why the energy dissipation rate of single F-actin networks can approach the performance of bundled F-actin networks with the increase of shear strain is (Fig. 2(d)). The result of energy separation of F-actin and crosslinkers under large strain conditions ( $\gamma=0.15\sim 0.2$ ) is consistent with our conclusions (see supplementary material, Fig. S5-S6).

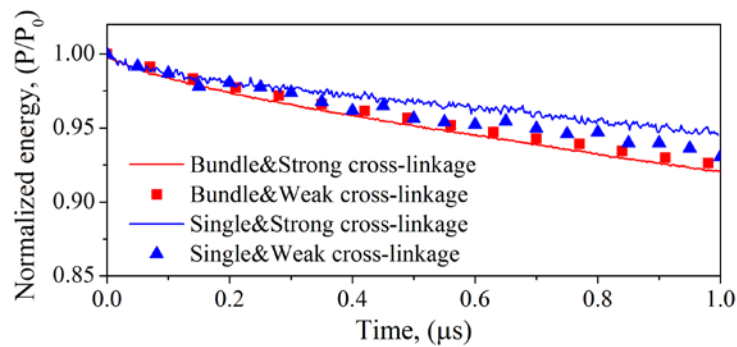
The potential energy distribution at a local cross-linkage in the network is extracted to illustrate the energy dissipation process in bundled F-actin networks with different cross-linking conditions, as shown in Fig. 4. After the crosslinkers are allowed to fail in the F-actin CSK, the potential energy will be efficiently released at the cross-linkage, which provides favorable conditions for living cells to perform flexible with respect to saturated mechanical inputs. Note that, there is still some residual potential energy at the cross-linkage, which is stored by the deformation of actin filaments and cannot be released immediately after the crosslinker breaks. However, without the constraints of crosslinkers, the F-actin network can gradually release this residual potential energy in longer duration by restructuring the cellular structures. This slow dynamics is caused by the time dependent behaviors of macromolecules and can take up to a few hours<sup>9</sup>. As introduced, this granular modelling strategy still keeps the high frequency oscillation of particles due to temperature ( $E_k = 3k_B T/2$ ,  $E_k$  is the kinetic energy,  $k_B$  is the Boltzmann constant and  $T$  is the absolute temperature), which weakens the ability of long-term prediction from the viewpoint of integration algorithm<sup>19</sup>. On the other hand, from physics, only thermal dynamic diffusion is usually insufficient for the network to overcome the barriers on energy landscape<sup>9</sup>, living cells still need non-thermal origin of energy (such as ATP dependent protein conformational changes) to overcome the energy barriers during the phase change<sup>7</sup>, which further limits the ability of long-term prediction of the granular modeling strategy in this letter. Therefore, we only track the transient changes of F-actin networks that are induced by transient crosslinker unbinding events instead of the long-term spontaneous responses. The degree of energy dissipation in single F-actin networks is not as significant as bundled F-actin networks. Please refer to Fig. S7 for further information of the energy distribution in single F-actin networks before and after crosslinker unbinding happens.





**Fig. 4** The contour of energy distribution in bundled F-actin networks under the circumstance of  $\gamma=0.05$ . The energy unit for this contour is kCal/mol.

Moreover, in order to investigate the effects of crosslinker strength, another group of modeling scenarios is designed in which the rupture force of crosslinkers is decreased to 100pN from the aforementioned limit of 180pN. The energy dissipation processes are still tracked under different strain conditions. Herein, we only provide the result of small deformation condition as an illustration, which has been shown in Fig. 5. The energy dissipation efficiency of single F-actin networks is increased when the fracture limit of crosslinkers is decreased. However, this change of rupture limit of crosslinker has no significant effects on the behaviors of bundled F-actin networks. This is because the stress-levels are different between bundled F-actin networks and single F-actin networks. Bundle F-actin networks exhibits less sensitivity to the crosslinker strength as the stress-level at cross-linkage is usually sufficient to break even tougher crosslinkers. However, for single F-actin networks, the stress-level could be lower than the critical rupture force. Therefore, the unbinding events can more easily happen with the decrease of crosslinker strength in single F-actin networks. It is therefore arguable that, the rate of energy dissipation in F-actin networks depends not only on the absolute value of crosslinker rupture force, but also on the hierarchical structures of actin filament in the network. It can be concluded that, for single F-actin network, the dependency of energy dissipation ability on crosslinker strength would be weakened with the increase of shear strain (i.e.  $\gamma=0.15\sim0.2$ ). This can be validated by modelling results under large strain conditions (see Figs. S8-S9 ).



**Fig. 5** The energy dissipation profile for both bundled and single F-actin networks when the crosslinker ultimate strength is reduced ( $\gamma=0.05$ ). The efficiency of energy dissipation in single F-actin networks is dramatically changed compared to bundled F-actin networks.

## 4. Conclusion

In summary, the mechanobiological behaviors of F-actin networks are dynamical performances that depend on both intrinsic material behaviors of actin and the physiological behaviors of various functional actin binding proteins. By using granular simulation technique, we have designed a highly coarse-grained model to understand the physical mechanisms of energy dissipation in F-actin cytoskeleton. Our investigation numerically validates the critical role that crosslinker protein plays in the process of phase changes of F-actin cytoskeleton with respect to physical forces. According to the numerical analysis, crosslinker protein in the F-actin cytoskeleton are dynamically unbinding or rebinding in response to mechanical conditions, which sensitively mediates the mechanical performances of F-actin cytoskeleton. The flexible responses of F-actin cytoskeleton induced by crosslinker unbinding events can help living cells to maintain a healthy phase while being exposed to constantly changing mechanical signals. This mechanosensing characteristic of F-actin cytoskeleton is significant to the mechanical adaptability of living cells.

## Acknowledgement

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## Conflict of Interest Statement

The authors, Tong Li, Ling Liu, Dean Hu, Adekunle Oloyede, Yin Xiao, Prasad Yarlagadda and YuanTong Gu, have no financial and personal relationships that could inappropriately influence or bias this work.

## Ethical Standards

No human/animal studies were carried out by the authors for this article.

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